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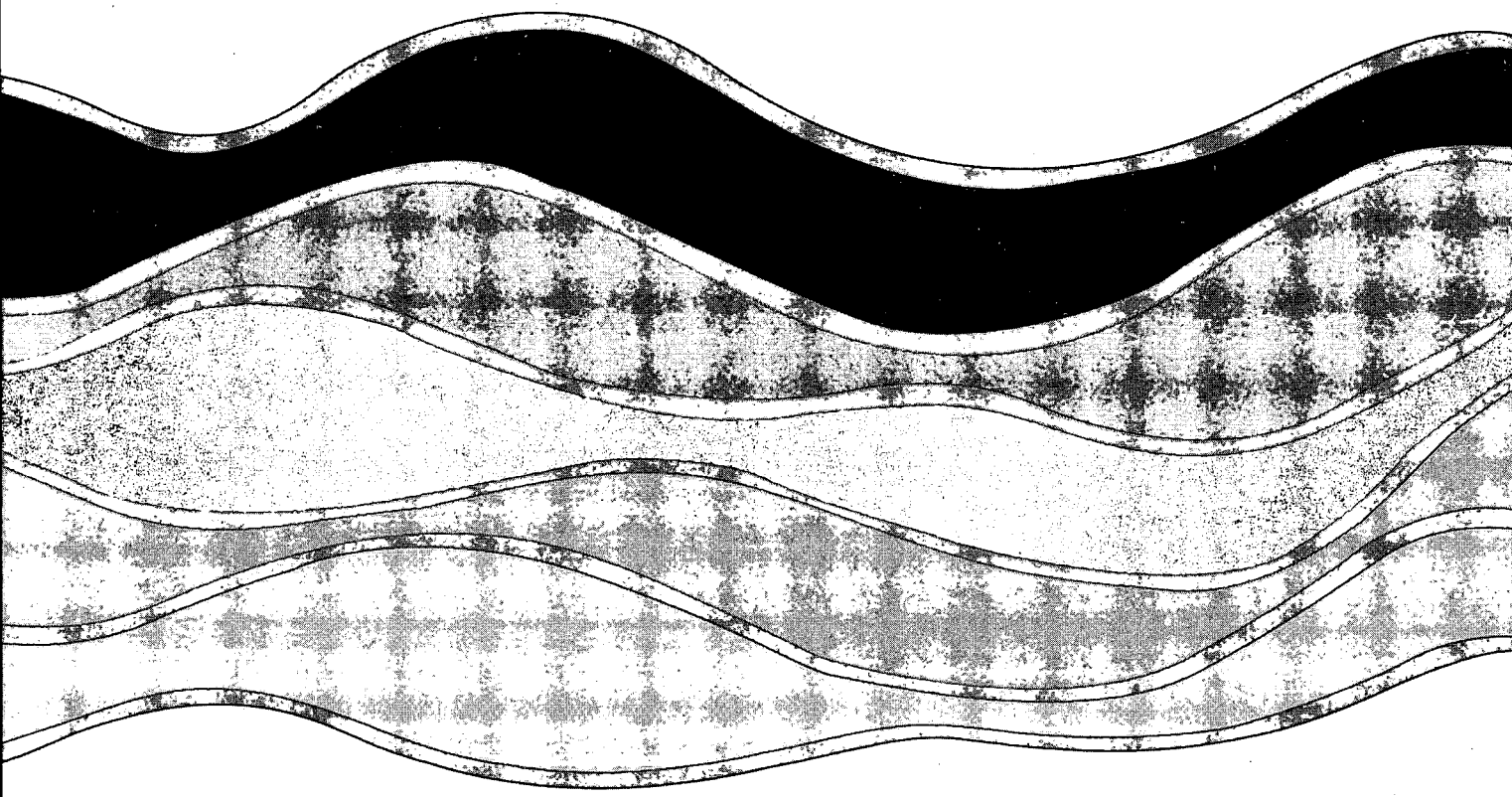

PREPARATION AND STABILITY OF CHLOROPHENOLS IN SEDIMENT EXTRACTS

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NWRI Contribution No. 94-62

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MANAGEMENT PERSPECTIVE

Concern about chlorophenol pollution in the aquatic environment has resulted in the monitoring of water, sediment and fish samples for phenols. To develop sediment extract reference samples fortified with chlorophenols as part of the range of QC samples used for evaluating laboratory performance in interlaboratory studies, considerable in-house investigation for the preparation and stability of chlorophenols in fortified sediment extracts was carried out. Results indicated that the chlorophenols in sediment extracts spiked at two concentration levels were stable for a minimum of three months when stored at 4°C or 25°C in the dark. This study provided the necessary information for designing and conducting an CEPA national interlaboratory study for analysis of chlorophenols in sediment extracts. An interlaboratory study further confirmed that chlorophenols in sediment extracts was stable over a period up to 6 months when the samples were preserved by stored at 4°C in the dark.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Les préoccupations concernant la pollution par le chlorophénol dans le milieu aquatique ont entraîné des activités de surveillance des phénols dans l'eau, les sédiments et des échantillons de poisson. Afin de préparer des échantillons de référence d'extraits de sédiments enrichis par des chlorophénols faisant partie de la gamme d'échantillons d'AQ utilisés pour l'évaluation du rendement des laboratoires dans le cadre d'études interlaboratoires, on a effectué d'importantes recherches à l'interne pour la préparation et l'obtention de chlorophénols stables dans les extraits de sédiments enrichis. Les résultats indiquaient que les chlorophénols dans les extraits de sédiments enrichis à deux niveaux de concentration étaient stables pour une période minimum de trois mois quand ils étaient stockés à l'obscurité à des températures de 4 ou de 25° C. Cette étude a fourni les informations nécessaires pour la conception et la mise en oeuvre d'une étude interlaboratoire nationale dans le cadre de la LCPE, destinée à l'analyse des chlorophénols dans les extraits de sédiments. En outre, une étude interlaboratoire a confirmé que les chlorophénols dans les extraits de sédiments étaient stables pour une période allant jusqu'à six mois quand les échantillons étaient préservés à l'obscurité, à une température de 4° C.

ABSTRACT

This report summarizes the results for the preparation and stability studies of sediment extracts fortified with six chlorophenols; namely, 2,4- and 3,4-dichlorophenols, 2,4,6- and 2,3,6-trichlorophenols, 2,3,4,6-tetrachlorophenol and pentachlorophenol. The analysis of chlorophenols in sediment extracts involved acetylation of chlorophenols with acetic anhydride, solvent extraction, silica gel cleanup of acetate derivatives and GC/ECD detection of the final extracts. Stability of chlorophenols in acetone-based sediment extracts was investigated under two storage conditions, namely 4°C and 25°C in the dark. Analytical results indicated no degradation of chlorophenols occurred in the two fortified sediment extracts (spiked with two concentration levels) for a minimum of 3 months.

The sample integrity of chlorophenols in sediment extracts was further confirmed by using these samples in an interlaboratory study. The results confirmed that no degradation occurred during the period of interlaboratory study (up to 6 months). For this interlaboratory study, participants were instructed to store these sample at 4°C in the dark until ready for analysis. Thus the stability of chlorophenols in sediment extracts was stable over a period up to 6 months when the samples were preserved by stored at 4°C in the dark.

RÉSUMÉ

Ce rapport résume les résultats d'études de préparation et de stabilité d'extraits de sédiments enrichis avec six chlorophénols, soit les 2,4- et les 3,4-dichlorophénols, les 2,4,6- et les 2,3,6-trichlorophénols, le 2,3,4,6-tétrachlorophénol et le pentachlorophénol. Pour l'analyse des chlorophénols dans les extraits de sédiments, on a procédé par acétylation des chlorophénols par l'anhydride acétique, l'extraction par des solvants, le nettoyage sur gel de silice des dérivés acétates et la détection par CG/DCE des extraits obtenus. On a étudié la stabilité des chlorophénols dans les extraits de sédiments à base d'acétone dans deux conditions d'entreposage, soit à 4 et à 25° C, à l'obscurité. Les résultats analytiques indiquaient qu'il n'y avait pas de dégradation des chlorophénols dans les deux extraits de sédiments enrichis (enrichis à deux niveaux de concentration) pendant une période minimum de trois mois.

De plus, l'intégrité des échantillons de chlorophénol dans les extraits de sédiments a été confirmée par l'utilisation de ces échantillons dans une étude interlaboratoire. Les résultats ont confirmé qu'il n'y avait pas de dégradation au cours de la période de l'étude interlaboratoire (jusqu'à six mois). Pour cette étude interlaboratoire, on a prescrit aux participants de stocker les échantillons à 4° C à l'obscurité jusqu'à l'analyse. Ainsi, la stabilité des chlorophénols dans les extraits de sédiments était adéquate pour une période atteignant jusqu'à six mois, quand les échantillons étaient préservés à 4° C, à l'obscurité.

1 INTRODUCTION

Chlorophenols are environmental contaminants especially in industrial wastewaters and sludges (1). Pentachlorophenol (PCP) has long been used as a wood preservative and other chlorophenols are often used as precursors in the production of many phenoxyalkanoic herbicides and biocides. Although application of PCP as a general wood preservative has now been generally banned and is limited to special applications such as wood preservative for hydro-poles, its acute toxicity and the previously wide applications will necessitate the continual monitoring of this compound in the environment for some considerable time. In addition, analysis for chlorophenols in sediment samples is particularly important because phenols are retained in large quantities by municipal solid wastes, landfill leachate and sediments (2-4).

From previous interlaboratory comparison studies, it was noted that many variations in extraction, cleanup and quantification of trace organic contaminants existed in sediment analysis. To develop sediment extract reference QC samples to evaluate laboratory performance for analysis of chlorophenols in sediments, it is necessary to carry out considerable in-house investigation for the preparation and stability of chlorophenols in sediment extracts. Thus in the present study, the stability of chlorophenols in sediment extracts over a period of 3 months was investigated under two storage conditions, namely 4°C and 25°C in the dark.

2 STUDY DESIGN

Several methods for chlorophenol analysis were developed by the formation of their acetate (5,6), chloroacetate (7) and pentaflurobenzyl ether (8) derivatives. For reasons of simplicity in derivatization, ruggedness and less interference, the acetate method was used in this study. A gas chromatograph (GC) with electron capture detection (ECD) was used to determine the selected

chlorophenols, namely 2,4 and 3,4-dichlorophenols (DCP), 2,4,6 and 2,3,6-trichlorophenols (TCP), 2,3,4,6-tetrachlorophenol (TeCP) and pentachlorophenol (PCP).

Two sediments selected for the preparation of sediment extracts were -200 mesh freeze-dried sediments SC-1 and LE-1, collected from Lake St. Claire and Lake Erie, respectively.

A period of about 3 months for the stability of chlorophenols in sediment extracts was investigated. No chemical preservative was added to the spiked sediment extracts. Most prepared sediment extracts in ampules were preserved by stored at 4°C in the dark. However, a small portions of ampules were stored at 25°C in the dark. The stability of chlorophenols in sediment extracts under these two storage conditions were compared.

3 EXPERIMENTAL

3.1 Standards and Reagents

All Chlorophenol standards were obtained from Aldrich Co. (Milwaukee, Wisconsin). Individual chlorophenol stock solutions of 1000 µg/mL were prepared. Acetic anhydride was purchased from BDH and triple-distilled before use.

3.2 Extraction, Fortification, Subsampling and Storage.

The protocol for the preparation of sediment extracts has been described in the procedure developed by Chau et al. (9). Two sediment extracts, namely SC-1 and LE-1 were prepared. For the spiking of sediment extracts, a stock mixed standard of six chlorophenols in acetone was prepared with the concentrations as follows: 2,4 and 3,4-DCP (200 µg/mL), 2,4,6 and 2,3,6-TCP (150 µg/mL), 2,3,4,6-TeCP and PCP (100 µg/mL). An appropriate amount of the above mixed standard was spiked into SC-1 and LE-1 sediment extracts, respectively , and made up to 500 mL with the respective sediment extract to give the

final concentrations of six chlorophenols as given in Table 1. In order to monitor the stability of chlorophenols in sediment extract at specified periods, and to correct for the variation of the instrument during these periods, a mixed standard solution of six chlorophenols was also simultaneously prepared from the same stock mixed standard (Table 1). All prepared standard and fortified sediment extract samples were thoroughly mixed and stored at 4°C, to achieve further equilibrium. A series of subsamples of standard solution and sediment extracts in ampules were prepared as follows. Aliquots of approximately 3 mL of the refrigerated samples were transferred into 5 mL precleaned ampules. The ampules were placed in groups of 20 or so and covered with aluminum foil while being cooled in a freezer at -10°C for at least 30 min. The ampules were sealed with a glass blowing torch immediately after being removed from the freezer.

All the ampules prepared (about 150 for each sample) were colour-coded and stored in the dark in a refrigerator at 4°C. For the stability study, about 30 ampules for each sample were also stored separately in the dark at room temperature (25°C).

3.3 Analytical Procedure

After the storage time had elapsed, four replicates of subsamples from the standard solution and sediment extracts were analyzed for chlorophenols by the analytical method described in a previous report (10). Briefly, the subsamples was brought to room temperature and exactly one mL of aliquot was transferred into a 250 mL Erlenmeyer flask containing 100 mL 1% K_2CO_3. Extractive acetylation of the chlorophenols was carried out by stirring the K_2CO_3 solution with 2 mL of acetic anhydride and 25 mL of petroleum ether for 30 min. This process was repeated twice and the combined petroleum ether layer was dried with anhydrous sodium sulphate and evaporated down to ca. 5 mL with a Snyder column in the presence of 2 mL of isooctane as a keeper. The petroleum ether layer was further evaporated to 2 mL using a gentle stream of nitrogen. A 5.0 cm 5% deactivated silica gel column was prepared with a 23 cm long disposable Pasteur pipet. The acetylation products were applied to the silica

gel column and the acetates of chlorophenols were collected by eluting the column with 10 mL of toluene. For each set of samples, an aliquot of the chlorophenol spiking solution was also derivatized, cleaned up and used as an external standard for instrument calibration.

For GC/ECD analysis, a 30 m x 0.25 mm i.d. OV-1 capillary column was used with the following temperature program : initial column temperature, 70°C, initial time, 0.5 min, oven temperature programming rate 1, 10°C/min (70° to 130°C), held at 130°C for 5 min, programming rate 2, 2°C/min (130° to 170°C), held at 170°C for 5 min. Carrier gas was helium and linear velocity was 25 cm/sec. A two µL aliquot was injected in the splitless mode by an autosampler with the splitless valve on for 0.5 min.

4.0 RESULTS AND DISCUSSION

Tables 2a and 2b present mean % recoveries of six chlorophenols in fortified sediment extracts following various storage times at 4°C in the dark for SC-1 and LE-1 sediment extracts, respectively. Before fortification, these two original sediment extracts showed no detectable amount of the six chlorophenols studied. The mean % recoveries of chlorophenols in fortified SC-1 sediment extract (at higher spiking concentration levels) were between 94 and 116% over 3 months study period and the relative standard deviations of replicate analysis were below 12% in most cases. These results were similar to those obtained for the stability study of preserved fish samples (10). For fortified LE-1 sediment extract (containing 1/4 the phenol levels), the mean % recoveries of chlorophenols were between 94 and 106% and the relative standard deviations were below 15%. No degradation of chlorophenols in the fortified sediment extracts was observed in sediment extracts at these two concentration levels.

It has been common practice to preserve sample integrity by storing samples at 4°C in the dark. However, to provide some additional information, the effect of storage at 25°C in the dark on the stability of chlorophenols in sediment extracts was also investigated for 3 months. The results are given

in Table 3. Overall, it shows no significant difference for recoveries of chlorophenols as compared with those stored at 4°C in the dark for both fortified sediment extracts.

To further confirm the long-term stability of chlorophenols in sediment extracts, the standard solution and fortified sediment extracts prepared for the present study (Table 1) were used as QC samples in an interlaboratory study (11). Comparison of the interlaboratory medians of chlorophenols with the design values showed that agreement for five out of the six chlorophenols was satisfactory (within ±25% of the design values) and only results for TeCP was more than 25% different from the design value. Similar results were obtained for sediment extract samples. The reason for these disagreements of TeCP results in standard solution and sediment extracts is not clear. Perhaps TeCP standard used for preparation of stock solution contained significant amounts of impurity. Thus percent recoveries of chlorophenols in sediment extracts were also calculated using interlaboratory medians (rather than the design values). Mean % recoveries of chlorophenols in fortified sediment extracts, based on the interlaboratory results, are given in Table 4. The results confirm that no degradation occurred during the period of interlaboratory study (up to 6 months). For this interlaboratory study, participants were instructed to store these sample at 4°C in the dark until ready for analysis. Thus the stability of chlorophenols in sediment extracts was stable over a period up to 6 months when the samples were preserved by stored at 4°C in the dark.

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Table 1. **Concentrations of chlorophenols in standard solution and fortified sediment extracts (All values are in µg/mL).**

Parameter	Standard solution	Fortified sediment extracts	
		SC-1	LE-1
2,4-DCP	10.0	10.0	4.0
3,4-DCP	10.0	10.0	4.0
2,4,6-TCP	7.5	7.5	3.0
2,3,6-TCP	7.5	7.5	3.0
2,3,4,6-TeCP	5.0	5.0	2.0
PCP	5.0	5.0	2.0

Table 2a. **Mean % recoveries of chlorophenols in fortified SC-1 sediment extract, stored at 4°C in the dark.**

Parameter	Month 1		Month 2		Month 3	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
2,4-DCP	112.3	3.5	100.1	9.7	107.7	2.1
3,4-DCP	112.4	3.8	99.7	9.7	101.7	1.5
2,4,6-TCP	115.0	3.7	94.7	9.8	107.3	2.6
2,3,6-TCP	114.3	3.9	96.9	10.9	103.1	2.1
2,3,4,6-TeCP	116.1	5.0	96.7	3.6	101.1	3.2
PCP	110.9	9.6	108.9	9.3	96.9	1.2

Table 2b. Mean % recoveries of chlorophenols in fortified LE-1 sediment extract, stored at 4°C in the dark.

Parameter	Month 1		Month 2		Month 3	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
2,4-DCP	97.4	3.3	98.5	9.4	105.5	7.0
3,4-DCP	96.5	3.2	99.0	11.4	95.0	6.1
2,4,6-TCP	98.2	3.0	99.6	11.2	101.0	13.2
2,3,6-TCP	97.4	2.7	91.1	6.1	97.8	12.5
2,3,4,6-TeCP	100.7	1.2	100.4	11.4	99.9	10.8
PCP	93.9	2.9	96.8	12.0	94.5	7.3

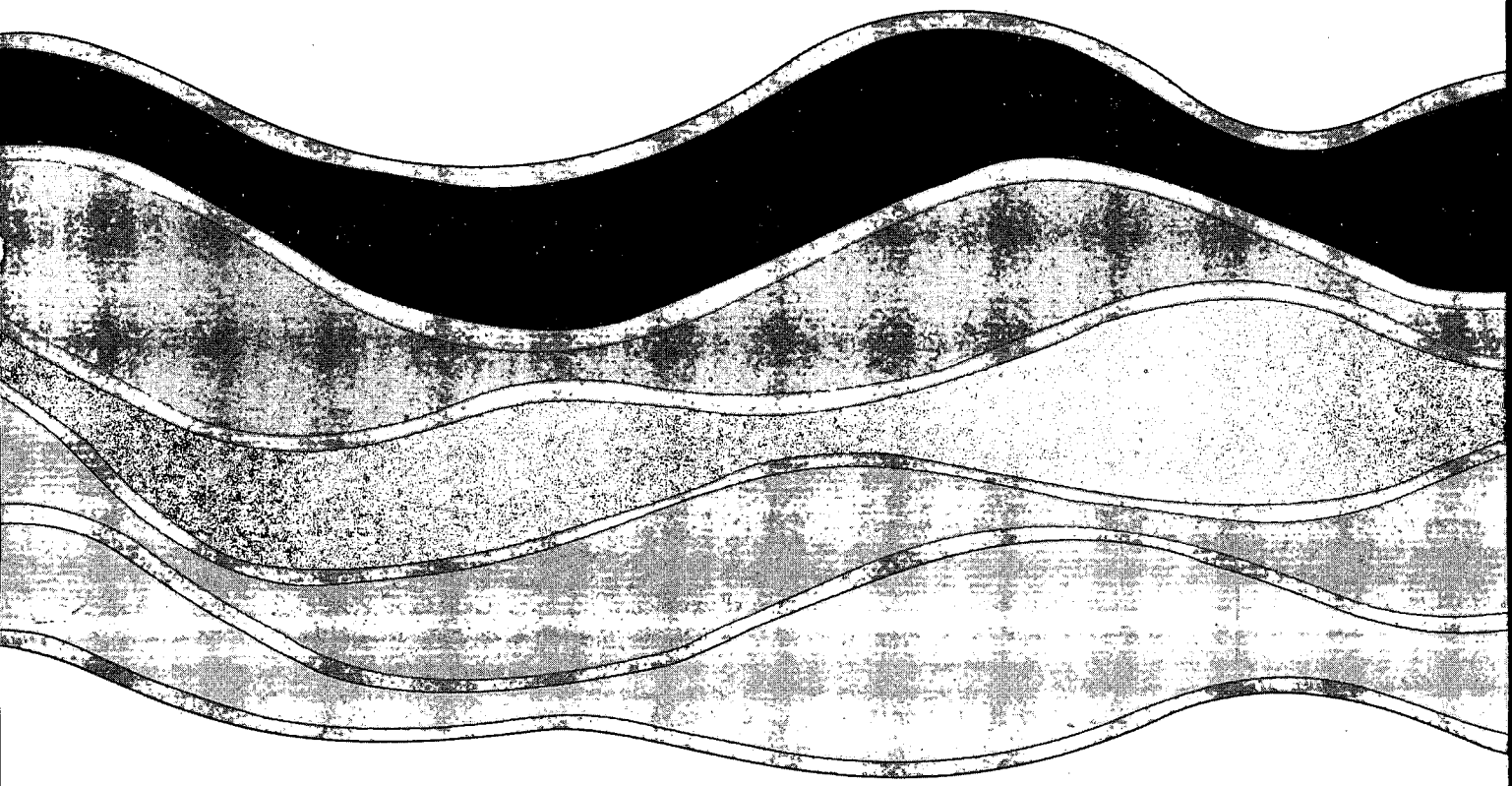
Table 3. Mean % recoveries of chlorophenols in fortified sediment extracts, stored at 25°C in the dark for 3 months.

Parameter	Fortified sediment extracts			
	SC-1		LE-1	
	Mean	S.D	Mean	S.D.
2,4-DCP	107.2	12.6	113.0	10.2
3,4-DCP	103.4	10.1	104.5	9.0
2,4,6-TCP	109.5	12.3	111.1	10.9
2,3,6-TCP	104.3	9.8	107.7	10.4
2,3,4,6-TeCP	107.1	14.3	106.7	10.6
PCP	104.0	11.0	104.9	8.1

Table 4. **Mean % recoveries of chlorophenols in fortified sediment extracts, based on interlaboratory results (study no. CP-1).**

Parameter	Fortified sediment extracts			
	SC-1		LE-1	
	Mean*	S.D.	Mean*	S.D.
2,4-DCP	93.7	2.5	101.2	0.4
3,4-DCP	93.0	3.3	95.1	5.6
2,4,6-TCP	108.1	7.3	105.8	2.5
2,3,6-TCP	107.8	4.2	108.1	10.9
2,3,4,6-TeCP	108.2	0	89.9	2.9
PCP	90.2	2.8	88.6	10.5

Note: * Average of interlaboratory median % recoveries of duplicate samples.



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